specification has been amended pursuant to the suggestion of the Examiner. Withdrawal of the objections is, therefore respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 3, 8, and 10 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. More specifically claim 3 has been rejected as being unclear in the recitation of "translocated." The term translocated has been deleted from claim 3. As such, the rejection has been obviated and withdrawal thereof is respectfully requested. Applicants further note for the record that "inversion" is, in fact, a form of amino acid "substitution." As such, the term "inversion" was redundant in claim 3.

Claims 8 and 10 have been rejected for recitation of "the nucleic acid of SEQ ID NO:2" with the notation that SEQ ID NO:2 is an amino acid sequence. Claims 8 and 10 have been amended, as requested by the Examiner to recite, SEQ ID NO:1. Withdrawal of the rejection is, therefore respectfully requested.

Claims 8 and 10 have been further rejected with the assertion that "hybridizes to" is indefinite without recitation of hybridization conditions. The Examiner further asserts that the

specification does not incorporate Maniatis et al. by reference. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The hybridization conditions incorporated into amended claims 8 and 10 are recited in Maniatis et al., page 9.52, under the protocol entitled "Hybridization or Radiolabeled Probes to Nucleic Acids Immobilized on Nitrocellulose Filters or Nylon Membranes." While specification does not specifically recite "incorporated by reference". Applicants believe the hybridization conditions would not the less be readily evident to one skilled in the art. Firstly, the specification recites that the methods of Maniatis et al. should be used in practicing the present invention. See for example page 9, line 15. Secondly, Applicants note that Maniatis et al. is considered to the "bible" of recombinant DNA/biotechnology protocols. One of ordinary skill in the art would look to Maniatis et al. for appropriate protocols/conditions in practicing the present invention. Applicants believe the hybridization conditions are effectively inherent to the invention, given the reference in the specification to use the protocols of Maniatis et al. when practicing the invention and given the singular prevalence that this reference has in the field of biotechnology as a source of protocols. As such, Applicants

believe it would not be new matter to incorporate the recited hybridization conditions into the claims. As such, withdrawal of the rejection is respectfully requested.

Rejections Under 35 U.S.C. § 102

Claims 3, 4, 8, 10, 15 and 16 have been rejected under 35 U.S.C. § 102 as being anticipated over Tsukamoto et al. or Yuuki et al.

The Examiner specifically asserts that "one α-amylase is equivalent in activity to another α-amylase unless the specific distinguishing characteristics are claimed." Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Enzymatic "activity" is characteristic of an enzyme. Specifically, the "activity" of an enzyme is a measure of the rate of substrate turnover per unit enzyme. Alfa-amylase optimally hydrolyzes the α-1,4-glucoside bond polysaccharides under alkaline pH conditions. As such, recitation that the enzyme is "equivalent in activity" to SEQ ID NO:2 is the recitation of a distinguishing characteristic of the enzyme, as requested by the Examiner. As indicated in the response filed on February 26, 1999, the α-amylases of Tsukamoto et al. and Yuuki et al. do not have the same

enzymatic activity as the α-amylases of SEQ ID NO:2 and thus any α-amylase encompassed by the present invention. The present invention is thus, not anticipated by either Tsukamoto et al. or Yuuki et al. and withdrawal of the rejection is respectfully requested.

As the above-presented amendments and remarks address and overcome the rejections of the Examiner, withdrawal of the rejections and reconsideration and allowance of the claims are respectfully requested. Should the Examiner have any questions regarding the present application, she is requested to contact MaryAnne Liotta, PhD (Reg. No. 40,069) in the Washington DC area, at (703) 205-8000.

Pursuant to 37 C.F.R. §§1.17 and 1.136(a), Applicants respectfully petition for a one (1) month extension of time for filing a response in connection with the present application and the required fee of $110.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees

required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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JAK/MAL/bsh
Attachment: Excerpt from Maniatis et al.

2

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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Preparation of Radiolabeled DNA and RNA Probes

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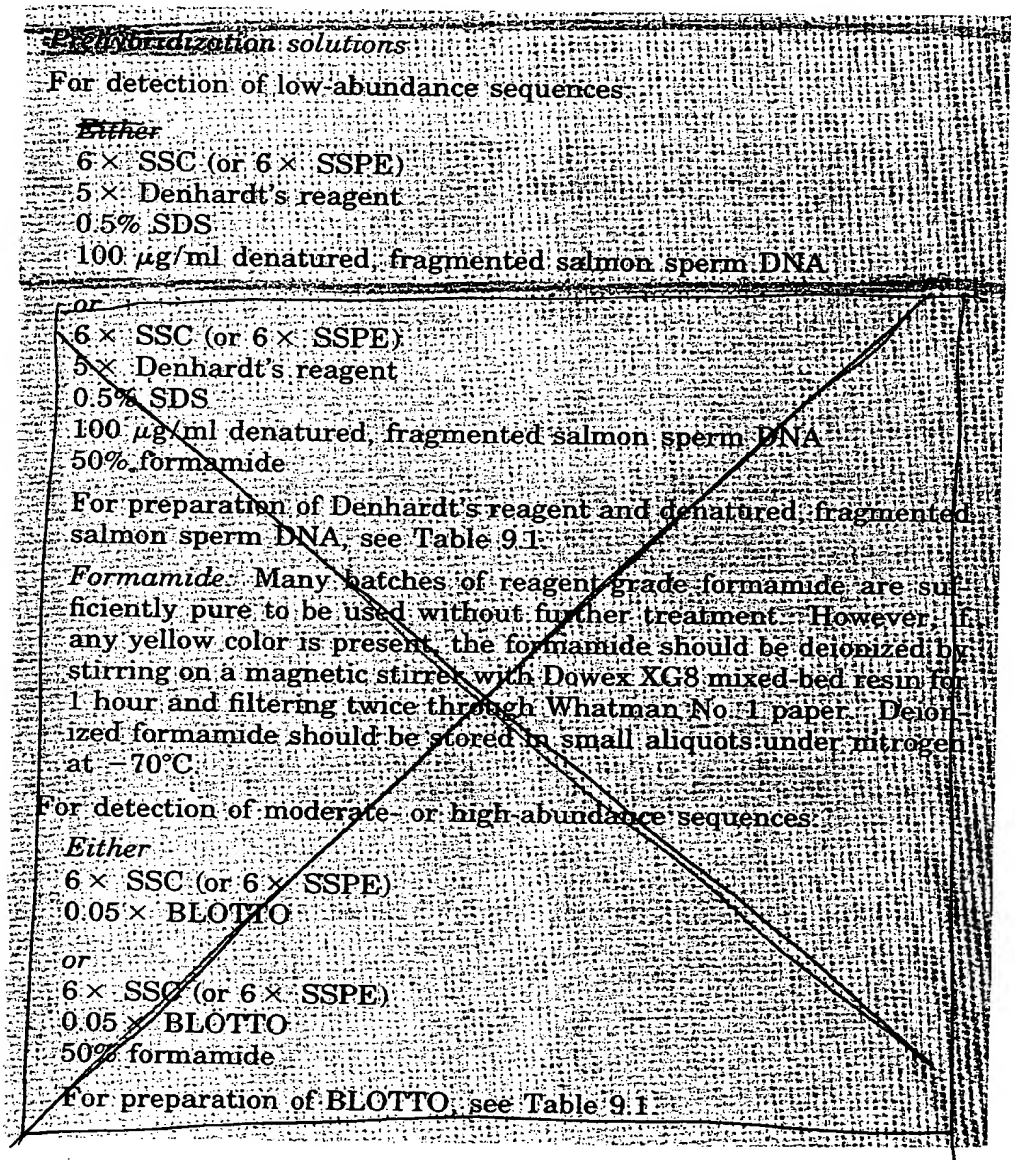
Hybridization of Radiolabeled Probes to Nucleic Acids Immobilized on Nitrocellulose Filters or Nylon Membranes

Although the method given below deals with RNA or DNA immobilized on nitrocellulose filters, only slight modifications are required to adapt the procedure to nylon membranes. These modifications are noted at the appropriate places in the text.

1. Prepare the prehybridization solution appropriate for the task at hand. Approximately 0.2 ml of prehybridization solution will be required for each square centimeter of nitrocellulose filter or nylon membrane.

   The prehybridization solution should be filtered through a 0.45-micron disposable cellulose acetate filter (Schleicher and Schuell Uniflow syringe filter No. 57240 or equivalent).



Prehybridization solutions

For detection of low-abundance sequences:

Either
6× SSC (or 6× SSPE)
5× Denhardt's reagent
0.5% SDS
100 μg/ml denatured, fragmented salmon sperm DNA

or
6× SSC (or 6× SSPE)
5× Denhardt's reagent
0.5% SDS
100 μg/ml denatured, fragmented salmon sperm DNA
50% formamide

For preparation of Denhardt's reagent and denatured, fragmented salmon sperm DNA, see Table 9.1.

Formamide. Many batches of reagent-grade formamide are sufficiently pure to be used without further treatment. However, if any yellow color is present, the formamide should be deionized by stirring on a magnetic stirrer with Dowex XG8 mixed-bed resin for 1 hour and filtering twice through Whatman No. 1 paper. Deionized formamide should be stored in small aliquots under nitrogen at −70°C.

For detection of moderate- or high-abundance sequences:

Either
6× SSC (or 6× SSPE)
0.05× BLOTTO

or
6× SSC (or 6× SSPE)
0.05× BLOTTO
50% formamide

For preparation of BLOTTO, see Table 9.1.

When ^{32}P-labeled cDNA or RNA is used as a probe, poly(A)$^+$ RNA at a concentration of 1 μg/ml may be included in the prehybridization and hybridization solutions to prevent the probe from binding to T-rich sequences that are found fairly commonly in eukaryotic DNA.

2. Float the nitrocellulose filter ~~or nylon membrane~~ containing the target DNA on the surface of a tray of 6× SSC (~~or 6× SSPE~~) until it becomes thoroughly wetted from beneath. Submerge the filter for 2 minutes.

3. Slip the wet filter into a heat-sealable bag (e.g., Sears Seal-A-Meal or equivalent). Add 0.2 ml of prehybridization solution for each square centimeter of nitrocellulose filter or nylon membrane.

   Squeeze as much air as possible from the bag. Seal the open end of the bag with the heat sealer. Incubate the bag for ~~1~~ 2 hours submerged at the appropriate temperature (68°C for aqueous solvents; ~~42°C for solvents containing 50% formamide~~).

   Often, small bubbles of air form on the surface of the filter as the temperature of the prehybridization solution increases. It is important that these bubbles be removed by occasionally agitating the fluid in the bag; otherwise, the components of the prehybridization solution will not be able to coat the filter evenly. This problem can be minimized by heating the prehybridization solution to the appropriate temperature before adding it to the bag.

4. If the radiolabeled probe is double-stranded, denature it by heating for 5 minutes at 100°C. ~~Single-stranded probe need not be denatured~~. Chill the probe rapidly in ice water.

   Alternatively, the probe may be denatured by adding 0.1 volume of 3 N NaOH. After 5 minutes at room temperature, transfer the probe to ice water and add 0.05 volume of 1 M Tris · Cl (pH 7.2) and 0.1 volume of 3 N HCl. Store the probe in ice water until it is needed.

   For Southern hybridization of mammalian genomic DNA where each lane of the gel contains 10 μg of DNA, 10–20 ng/ml radiolabeled probe (sp. act. = 10^9 cpm/μg or greater) should be used. For Southern hybridization of fragments of cloned DNA where each band of the restriction digest contains 10 ng of DNA or more, much less probe is required. Typically, hybridization is carried out for 6–8 hours using 1–2 ng/ml radiolabeled probe (sp. act. = 10^9 cpm/μg or greater).

5. Working quickly, remove the bag containing the filter from the water bath. Open the bag by cutting off one corner with scissors. Add the denatured probe to the prehybridization solution, and then squeeze as much air as possible from the bag. Reseal the bag with the heat sealer so that as few bubbles as possible are trapped in the bag. To avoid radioactive contamination of the water bath, the resealed bag should be sealed inside a second, noncontaminated bag.

   When using nylon membranes, the prehybridization solution should be *completely* removed from the bag and immediately replaced with hybridization solution. The probe is then added and the bag is resealed.

Hybridization solution for nylon membranes

6× SSC (or 6× SSPE)
0.5% SDS
100 μg/ml denatured, fragmented salmon sperm DNA
50% formamide (if hybridization is to be carried out at 42°C)

6. Incubate the bag submerged in a water bath set at the appropriate temperature for the required period of hybridization.

68°C 8hr

7. Wearing gloves, remove the bag from the water bath and immediately cut off one corner. Pour out the hybridization solution into a container suitable for disposal, and then cut the bag along the length of three sides. Remove the filter and immediately submerge it in a tray containing several hundred milliliters of 2× SSC and 0.5% SDS at room temperature.

   Important: Do not allow the filter to dry out at any stage during the washing procedure.

8. After 5 minutes, transfer the filter to a fresh tray containing several hundred milliliters of 2× SSC and 0.1% SDS and incubate for 15 minutes at room temperature with occasional gentle agitation.

   If short oligonucleotides are used as probes, washing should be carried out only for brief periods (1–2 minutes) at the appropriate temperature. For a discussion of the stability of hybrids involving oligonucleotides, see Chapter 11.

9. Transfer the filter to a flat-bottom plastic box containing several hundred milliliters of fresh 0.1× SSC and 0.5% SDS. Incubate the filter for 30 minutes to 1 hour at 37°C with gentle agitation.

10. Replace the solution with fresh 0.1× SSC and 0.5% SDS, and transfer the box to a water bath set at 68°C for an equal period of time. Monitor the amount of radioactivity on the filter using a hand-held minimonitor. The parts of the filter that do not contain DNA should not emit a detectable signal. You should not expect to pick up a signal on the minimonitor from filters containing mammalian DNA that has been hybridized to single-copy probes.

11. Briefly wash the filter with 0.1× SSC at room temperature. Remove most of the liquid from the filter by placing it on a pad of paper towels.

12. Place the damp filter on a sheet of Saran Wrap. Apply adhesive dot labels marked with radioactive ink to several asymmetric locations on the Saran Wrap. These markers serve to align the autoradiograph with the filter. Cover the labels with Scotch Tape. This prevents contamination of the film holder or intensifying screen with the radioactive ink.

    Radioactive ink is made by mixing a small amount of ^{32}P with waterproof black drawing ink. We find it convenient to make the ink in three grades: very hot

(>2000 cps on a hand-held minimonitor), hot (>500 cps on a hand-held minimonitor), and cool (>50 cps on a hand-held minimonitor). Use a fiber-tip pen to apply ink of the desired hotness to the adhesive labels. Attach radioactive-warning tape to the pen, and store it in an appropriate place.

13. Cover the filter with a second sheet of Saran Wrap, and expose the filter to X-ray film (Kodak XAR-2 or equivalent) to obtain an autoradiographic image (see Appendix E). The exposure time should be determined empirically. However, single-copy sequences in mammalian genomic DNA can usually be detected after 16–24 hours of exposure at −70°C with an intensifying screen.